A QUICK AND INEXPENSIVE METHOD FOR MAKING TEMPORARY SLIDES OF LARVAL CHIRONOMIDAE (DIPTERA)[1]

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ABSTRACT: Glycerine is used to mount chironomid larvae on slides, under separate cover slips, for rapid and accurate identification. Glycerine is an inexpensive substitute for water-based media such as CMC-10.

It is not unusual to collect hundreds or thousands of chironomid larvae during a study of rivers, lakes, or streams. Mounting great numbers of larvae on slides soon becomes expensive because of the time involved and the supplies needed: slides, cover slips, mounting media, and solvents. Here we describe a technique that is both fast and inexpensive, the glycerine method. This method is most suitable for tabulating species and instar data on known species, as in life history studies where larvae are routinely collected from the same habitats.

Many workers use resinous media, such as Canada balsam and Euparal, to mount chironomid larvae on slides. However, some workers are switching to water-based media because of the time involved in preparation of slides. Using water-based media also reduces the cost of supplies; no special solvents are needed, larvae can be mounted directly from water or alcohol, and slides and cover slips can be reused after washing. Glycerine (glycerol) is an inexpensive, easily obtainable substitute for commercial water-based media such as the popular CMC-10 (Klemm 1980). For an equivalent amount, lab grade glycerine costs less than half as much as CMC-10 and is available from several supply houses such as Carolina Biological Supply.

Although glycerine has been used for making temporary slides for many years (Peterson 1964), we are not aware that it has been used for slide-mounting chironomid larvae. Glycerine has the advantage of yielding clearly observable slide-mounts which are ready for immediate examination under the dry field microscope. It can easily be removed from slides, and glycerine goes a long way. If a single larva (1-4 mm in body length) can be mounted in one spot of glycerine, then at least 300 larvae can be mounted in 1 ml of glycerine. Disadvantages of glycerine are that slides must be examined within a couple of days after mounting and glycerine does not clear specimens. Heavily sclerotized, dark, or large larvae can be cleared by digesting them in hot or cold 10% KOH before mounting.

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Glycerine Method

We store larvae in vials containing 80% ethanol. Before we remove larvae from the vials, we draw off the ethanol with an eyedropper and fill the vial with distilled water. We then pour the larvae into a dish that contains distilled water. Next we put 6 to 8 spots of glycerine on a clean 3 x 1 in slide, pick up each larva with a dissecting pin, and place it in one spot. When the larva comes into contact with the glycerine, slight shriveling of the body occurs. Next we place a 10 or 12 mm circular cover slip (1½ thickness) on each larva, and apply slight pressure. Larvae are rotated into proper position by moving the cover slips.

Specimens can be examined immediately under the dry field microscope. Glycerine remains slippery unless dry, and before immersion oil can be used slides must be dried. Otherwise the larva will move under the pressure of the objective lens on the cover slip. Air drying slides takes about 48 hrs; oven drying at 45° C takes about 30-45 min. Specimens should be examined before the glycerine crystallizes, about 48 hrs. after drying.

Using this procedure we can slide-mount about 200 larvae in one hour. It's possible to slide-mount more larvae by placing several larvae under larger cover slips. However, this alteration may interfere with the identification of the larvae since it is difficult to properly orient more than one larva under a single cover slip.

Soaking slides in tap water for 24 hrs will clean the slides unless immersion oil has been used. Then, detergent should be added to the water. Slides are removed one by one, wiped with a soft cloth or brush, rinsed, and placed flat in paper towels to dry. We transfer cover slips to a small dish of clean water, then place them separately on paper towels to dry. Larvae are discarded with the wash water.

If we select larvae mounted in glycerine for inclusion in the reference slide collection, we mount them in Euparal according to the following procedure. Place a few drops of distilled water around the edge of the cover slip until the glycerine becomes slippery and the cover slip becomes loose. Lift off the cover slip; place the larva in distilled water for about 5 min; transfer the larva to 95% ethanol for about 5 min; mount the larva directly into a spot of Euparal on a slide. In the distilled water the body contents empty out of the larval body and the head capsule. Although this makes the specimen delicate, there is no need to clear the larva in KOH and an excellent slide is produced.

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